We hypothesize that *in vitro* cultured CTL without RA lack the ability to cause GVHD in part due to deficient LPAM expression. We used an established murine GVHD model in which B6SJL Sp/LN cells were stimulated against DBA splenocytes with IL-2 and IL-7 with and without addition of RA (100 nm). Day 14 comparison of CTL and CTLRA revealed comparable CD4 and CD8 populations. CTL with and without RA showed CD8 LPAM expression of 58% and 0.8% and CD8 CCR9 53% and 10% respectively. *In vitro* cytotoxicity was comparable between CTL and CTLRA: 41% vs 51% (n = 3, P = .30). Both CTL groups had comparable *in vitro* migration towards SDF (P = ns) but CTLRA had increased migration towards TECK; 17.3% vs 4.6% (n = 4, P = .01). For *in vivo* homing, 10^7 labeled cells from each CTL with (CFSE) and without RA (TRITC) were coinjected intravenously and analysed after 16 hours. CTLRA had increased homing to Peyers patch and MLN compared to CTL without RA [homing index (CTLRA/CTL) 2.3 and 2.5 respectively]. This finding is exaggerated in the radiated host [homing index (CTLRA/CTL) 15 for PP and 11 for MLN]. CTL and CTLRA (5×10^6 cells each) were injected intravenously with or without C57BL/6J BM into irradiated (600 rads) B6D2F1 recipients (6 groups; radiation control, CTL, CTLRA, BM control, CTL + BM, CTLRA + BM). Mice were followed for clinical GVHD scores and CBC and histopathologic GVHD scores (liver, skin, lung, small and large intestines) were obtained. Both CTL groups without BM rescue developed lethal BM aplasia around day 24; however, histopathologic GVHD scores were similar (Table 1). CTL and CTLRA groups with BM rescue had full hematopoietic recovery, yet had similar histopathologic GVHD scores (BM control; 3.9, CTL + BM; 5, CTLRA + BM: 3.9, P = ns). Our data demonstrate that both CTL and CTLRA cause a lethal hematopoietic GVH reaction which could be abrogated by parent BM. Despite high LPAM and CCR9 expression, significant *in vitro* migration to TECK and *in vivo* homing to gut associated lymphoid tissues, RA treated CTL did not cause significant GVHD in gut, liver or skin. This suggests that defective gut homing alone may not be sufficient to explain the attenuated GVHD from cultured CTL (Table 1).

Table 1. *Data from Day 24 Sacrifice*

Parameter (Mean)	Radiation Control	CTL	CTLRA	*P* Value*
Hb (g/dL)	12.2	3.8	3.1	.0001
WBC × 10^6/L	1000	300	300	.07
Platelets × 10^3/L	667 200	50 200	29 500	.003
Combined GVHD histology score	4.9	4.4	4.2	.30

*CTL or CTLRA vs radiation control group.

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EARLY TREATMENT WITH CD4+CD25+ REGULATORY T CELLS PROVIDES PROLONGED SUPPRESSIVE EFFECTS WHICH CONTROL EVOLVING BUT NOT ESTABLISHED GRAFT-VERSUS-HOST-DISEASE

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In our previous study with Treg trafficking, bioluminescence imaging (BLI) indicated a persistence of signal consistent with a prolonged survival of Treg in vivo following allogeneic bone marrow transplantation. In the current study, we evaluated the duration of Treg suppression and the impact of Treg on evolving and established GvHD. Lethally irradiated Balb/c (H2d) hosts received 5×10^6 T-cell depleted bone marrow cells from wild-type FVB mice (H2q) on day 0. On day 2, 3×10^6 splenocytes, or 1×10^6 purified CD4+/CD8+ T cells (Tcon) from luciferase+ transgenic mice (FVB) were infused to induce GvHD. Treg, purified from wt-FVB mice, in a 1:1 dose ratio with Tcon, were infused either on day 0, 2, 9, or 23 post-transplantation. BLI was used to localize and quantify the proliferation of Tcon in the absence or presence of Treg. Signal intensity, measured by photons/second/mouse, was significantly decreased in animals which received Treg at day 0, 2, or 9 ($P < .05$). Importantly, the greatest reduction in signal intensity occurred when Treg were given prior to the induction of GvHD by Tcon. This reduction was associated with a significantly lower clinical score for GvHD. Studies in which Treg are given up to 10 days prior to the addition of Tcon show similar findings. At day 23, when clinical GvHD was fully established in mice which received Tcon, the addition of Treg did not alter the increasing BLI signal level or the clinical course such that all animals died of GvHD (P = .38). Lymphoid reconstitution was not affected by the addition of Treg prior to the induction of GvHD. In dose titration studies whereby Treg are given two days prior to the induction of GvHD, a 10-fold dose reduction in Treg was sufficient to significantly reduce Tcon proliferation and suppress clinical GvHD. We next assessed the duration of Treg suppressive effect by inducing GvHD on day 7, 14, 19 with luc+ Tcon following the infusion of Treg on day 0 of allogeneic BMT. Treg provided protection from the Tcon challenge at all 3 time points, leading to improved survival ($P < .05$). We conclude that Treg provide prolonged protection due to their ability to proliferate in vivo in an allogeneic setting, permitting a significant reduction in the number of Treg needed for adoptive transfer to induce a clinical response. In addition, we conclude that Treg suppress the early proliferation of Tcon, allowing them to prevent and control evolving but not established GvHD.

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INTERLEUKIN-2 (IL-2) AND GRANULOCYTE-MACROPHAGE STIMULATING FACTOR (GM-CSF) FOR TREATMENT OF RELAPSE AFTER ALLOGENEIC STEM CELL TRANSPLANT (ASCT)

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Donor lymphocyte infusion (DLI) is commonly used for relapse after allogeneic stem cell transplant (ASCT). Immune activation with cytokines is an alternative to DLI. We report a retrospective analysis and first 6 patients (pts) of a prospective study evaluating the safety and efficacy of granulocyte-macrophage stimulating factor (GM-CSF) and interleukin-2 (IL-2) administered at the time of relapse after ASCT in pts with hematologic malignancies. Pts received subcutaneous GM-CSF at 250 μg/day on days 1-14 and IL-2 at 1×10^6 units/m^2/day on days 8-14. Pts were off of immunosuppressive therapy and had no prior history of graft versus host disease (GVHD) at the start of treatment. A total of 10 pts have received cytokine therapy with IL-2/GM-CSF for treatment of relapsed AML (6), ALL (2), CML (1), MDS (1). Median age was 45 (range 8-61). Stem cell source included: peripheral blood = 7, bone marrow = 2, umbilical cord blood (UCB) = 1. Donor sources were matched-related sibling = 3, matched-unrelated donor = 7 (UCB = 1). Seven pts had resistant relapse or primary resistant disease at time of ASCT. Median time from transplant to relapse was 4 months (range = 1-14 months). Two pts had failed DLI and 5 pts had received reinduction chemotherapy prior to IL-2/GM-CSF. Seven pts responded to IL-2/GM-CSF (CR = 6, PR = 1). Two pts remain disease free at 18 and 26 months post IL-2/GM-CSF. Six pts developed GVHD (4/6 responders). Two pts had GM-CSF discontinued due to increase in peripheral blood blasts. No other toxicities were related to IL-2/GM-CSF except for flu-like symptoms and bone pains. In conclusion, cytokine therapy with IL-2/GM-CSF is feasible and we continue accrual to determine if this cytokine regimen is an alternative to DLI for relapse after ASCT.

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ELEVATED B CELL ACTIVATING FACTOR (BAFF) IN PATIENT PLASMA AFTER ALLOGENEIC STEM CELL TRANSPLANTATION IS A POTENTIAL BIOMARKER FOR CHRONIC GRAFT VERSUS HOST DISEASE

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Previous studies have identified a highly significant correlation between specific antibody responses directed against recipient